Characteristics of Mozzarella cheeses made with fortified milk or nonfat dry milk

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Concern over the possible use of substitutes for natural cheeses in federal food programs initiated our study of the textural properties of part skim Mozzarella cheese and Mozzarella substitutes. Cheeses were made from fresh milk (9% solids) or fresh milk fortified with 1 or 2% added calcium caseinate (CC) or nonfat dry milk (NFDM). Cheeses made from milk containing 9, 10, or 11% solids were also made using only reconstituted NFDM and cream. All cheeses showed a significant ($p<0.0001$) decrease in α_{S1}-casein content during 6 wk of storage at 4°C. As a result, mean values from meltability, texture profile analysis, small amplitude oscillatory shear measurements, and torsion analyses all changed significantly with storage. Almost all cheeses tended to become more rubbery during the storage period. This study showed that CC and NFDM can be added to Mozzarella cheese milk at a concentration of 1 or 2% without affecting the final properties of the cheese.

51 **Mozzarella cheese** (technology) 51 **Mozzarellakäse** (Technologie)

1. Introduction

Federal food programs in the USA utilize large quantities of natural cheeses. Consumer interest in lowfat cheese and manufacturer interest in utilizing economical dairy-based ingredients in cheese production have raised the possibility of the use of cheese substitutes in food programs (4). Cheese substitutes or imitation cheeses are made from either fresh milk with added calcium caseinate (CC) or nonfat dry milk (NFDM), or reconstituted NFDM with added vegetable oil. Currently, genuine cheese products may be made from NFDM, but not caseinates (24).

A record 1.075×10^9 kg of Mozzarella cheese was produced in the US in 1998 (23), mainly due to its use for pizza; this requires specific textural and functional properties. Concern over the possible reduction in functionality if cheese substitutes were to be used on school lunch pizza prompted our investigation into the differences in rheological properties of part-skim Mozzarella cheese and Mozzarella substitutes. A few studies have been made on the functional properties of Mozzarella cheese products made with NFDM or added caseinate. Low fat, high moisture Mozzarella made with 10% reconstituted NFDM (only level reported) and cream was reported to be comparable in meltability, stringiness, and browning to cheese made with fresh milk (2). The yield of cheese was reportedly increased with the addition of CC or NFDM to the milk (13). However, problems such as slower rennetability, longer coagulation time, and slower syneresis may also be encountered (8). The addition of 1 or 2% CC to cheese milk alters the fat distribution within part skim Mozzarella cheese causing significant changes in the melting properties (17). Reconstitution of up to 3% NFDM in Mozzarella cheese milk did not significantly alter the texture, meltability, or free oil formation in the cheese, although apparent viscosity was higher (25).

The textural properties of cheese can be examined using a variety of instruments (22). Texture profile analysis (TPA) is a force-compression test which produces values for hardness and cohesiveness. Small amplitude oscillatory shear instruments measure storage modulus (G'), loss modulus (G"), and complex viscosity (η^*), which give information on the elastic and viscous properties of the sample in the linear viscoelastic range. Recent improvements in instrumental torsion (twisting) testing for meat emulsions (5) offer a different approach to cheese rheology using shear stress and shear strain at the point of failure.

The object of this study was to compare the rheological properties of part-skim Mozzarella cheese to products made from fresh milk fortified with CC or NFDM, or made from reconstituted NFDM.

2. Materials and methods

2.1 Cheesemaking

Part skim Mozzarella cheeses were prepared in a random design using 22.7 kg of milk per batch. Two different batches were prepared on any day from the same milk, and 2 replicates of each cheese type were made. Mixed herd milk from a local agricultural college was standardized to 2.3% fat and was pasteurized at 63°C for 30 min. Milk consisted of either fresh skim milk (the control, containing 9% solids-not-fat), fresh skim milk with 1 or 2% added CC (New Zealand Milk Products, Santa Rosa, CA, USA), fresh skim milk with 1 or 2% added NFDM (Dairygold, Seattle, WA, USA or Dairy America, Dublin, CA, USA), or reconstituted NFDM (9, 10, or 11% solids-not-fat). Allowances for the fat content of the NFDM were made. Following overnight storage at 4°C, the cheesemilk was heated to 33°C and Mozzarella was prepared as previously described (7). The curd was cooked to 40°C in 60 to 70 min with stirring. The reconstituted NFDM cheeses were cooked to 43°C since cooking at 40°C resulted in overly rapid pH development, an excess of moisture in the curd, and a very soft cheese.

2.2 Compositional analyses and yield

Moisture content of the cheeses was determined by the forced-draft oven method (1) and fat content was determined by the modified Babcock method (9). Both analyses were performed in triplicate after 2 or 3 d of storage. Moisture in nonfat substance (MNFS) was calculated as 100 x (% moisture)/(100 - % fat). Similarly, fat in dry matter (FDM) was calculated as 100 x (% fat)/(100 - % moisture). Percentage yield of cheese was calculated as 100 x (kg cheese)/(22.7 kg milk).

2.3 Proteolysis

To follow the breakdown of α_{S1}-casein during storage, cheeses were extracted at 1 and 6 wk, as described previously (21). Extracts were analyzed by SDS-PAGE using the PhastSystem (Amer. Pharmacia Biotech, Piscataway, NJ, USA) with 20% homogeneous Phast gels. Image analyses of gel bands were performed with the ImageQuaNT program of a Molecular Dynamics Personal densitometer SI model 375A (Molecular Dynamics, Sunnyvale, CA, USA). Bands used for calculations included α_{S2}-, α_{S1}-, α_{S1}-I, β-, γ_1-, para κ-, and γ_2-, and γ_3-caseins. Results are reported as the percentage of α_{S1}-casein band area in the total of all above band areas.

2.4 Meltability and rheology

At 1 and 6 weeks after brining, cheese samples were removed from refrigerated storage and allowed to stand at room temperature for 1 h prior to testing.

Meltability was determined in triplicate as previously described (19) by the Schreiber test. Texture profile analyses were conducted as previously described (20) with an Instron Universal Testing Machine model 4201 (Instron, Inc., Canton, MA, USA) to determine TPA hardness (in newtons) and TPA cohesiveness (unitless). Small amplitude oscillatory shear measurements were performed as previously described (20), with G', G", and η^* being determined with a Rheometrics Dynamic Analyzer model RDA-700 (Rheometrics Scientific, Piscataway, NJ, USA) at 22°C. The values for G' and G" (in kPa), and for η^* (in kPa·s) were then obtained at the selected frequency of 100 rad/s.

Cheese samples were also tested in a torsion gelometer (Gel Consultants, Raleigh, NC, USA) to obtain shear stress and shear strain at the point of fracture. Four sample plugs were bored from the block and milled to the appropriate shape as described by HAMANN (6). The Gelometer was operated at 2.5 rpm and recorded shear stress and shear strain in kPa, from which shear rigidity (unitless ratio of shear stress to shear strain) was calculated.

2.5 Statistical analyses

The data were analyzed using General Linear Models and correlation procedures (16). Means comparisons were performed using Duncan's multiple range test. Differences are described as significant only when $p<0.05$.

3. Results

3.1 Cheese composition and yield

The compositional data of the cheeses prepared from fresh milk (control milk or with 1 or 2% added CC or NFDM) are shown in Table 1. These cheeses had similar moisture levels, and all had final pH levels of 5.2 to 5.3. Cheese made with added CC had significantly lower fat and FDM than the control cheese; cheese made with the 2% added CC was the only cheese that contained less than 30% FDM.

Table 1: Moisture, fat, moisture in nonfat substance (MNFS), fat in dry matter (FDM), and yield of Mozzarella cheeses prepared with calcium caseinate (CC) or nonfat dry milk (NFDM)[1] (%)

Cheese type	H_2O	Fat	MNFS	FDM	Yield
Fresh milk cheese cooked at 40°C					
Control	51.6	18.1	62.9	37.3	10.4
1% CC added	52.5	15.1	61.8	31.8	12.8
2% CC added	53.0	12.6	60.7	26.8	15.5
1% NFDM added	52.3	16.7	62.7	34.9	11.8
2% NFDM added	52.5	15.6	62.2	32.8	12.3
MSD[2]	1.7	1.4	1.1	1.9	1.0
Reconstituted milk cheese cooked at 43°C					
9 % NFDM	49.4	18.6	60.7	36.7	10.1
10% NFDM	50.0	18.2	61.1	36.4	10.5
11% NFDM	50.9	16.4	60.8	33.3	11.1
MSD[2]	2.1	1.7	2.0	2.7	1.6

[1]Averages of 2 replicates; [2]minimum significant difference

Table 1 also shows the compositional data for the cheeses made from reconstituted milk. The moisture and MNFS levels of these 3 cheese types were similar, but cheese made from reconstituted milk containing 11% NFDM had lower fat and FDM levels than the other 2 cheese types. These cheeses also had final pH levels of 5.2 to 5.3.

Syneresis of whey in cheesemaking may be impaired by addition of milk solids (15), which bind water. In our study, the moisture contents of the cheeses made from fortified milk were not significantly different from the control. The composition of the cheeses fit within the US definitions for low moisture part skim Mozzarella (24), with the exception of the cheeses made from milk with 2% added CC. This cheese type gave a significantly higher yield than any of the other types, but the FDM level was low, which could result in undesirable texture and flavor properties. A cheesemaker would have to start with milk containing more fat to compensate for the extra protein in a 2% added CC cheese (8).

Cheese yield data are also included in Table 1. Cheese made from milk with 2% added CC showed a significantly higher yield than all the other cheeses. The yield in the cheeses containing up to 2% added CC or NFDM was inversely related ($p<0.0005$) to fat and FDM with correlation coefficients (R) of −0.86 and −0.81, respectively. The yield of these cheeses was directly related ($p<0.005$) to moisture, but the correlation coefficient was only 0.56. The increase with MNFS was not as significant.

3.2 Proteolysis

The percentage of α_{s1}-casein in each cheese decreased significantly ($p<0.0001$) during 6 wk of refrigerated storage (Table 2). Chymosin (rennet) hydrolyzes κ-casein, inducing coagulation, and residual rennet in the curd breaks down α_{s1}-casein, the main structural protein in cheese (3). Initial chymosin cleavage between phe23-phe24 in α_{s1}-casein is followed by cleavages at the carboxyl terminal (14). Monitoring the loss in percentage of α_{s1}-casein samples at a given week, therefore, provides an estimate of the extent of α_{s1}-casein proteolysis as affected by storage time, differences in composition, pretreatments, or other processing differences. In this study, α_{s1}-casein proteolysis was not affected by solids content.

Table 2: Mean values of α_{s1}-casein, meltability, and rheological properties of Mozzarella cheeses prepared from fresh milk or reconstituted milk, after 1 and 6 wk of refrigerated storage

Parameter	Fresh milk cheeses			Reconstituted milk cheeses		
	1 wk	6 wk	MSD[1]	1 wk	6 wk	MSD
α_{s1}-casein, %	27.2	8.4	1.9	28.7	13.3	2.0
Meltability	2.08	1.87	0.19	1.98	1.71	0.16
Hardness, N	63.6	40.4	15.1	90.1	74.8	13.2
Cohesiveness	0.62	0.47	0.05	0.48	0.31	0.05
G', kPa	55.2	42.2	11.4	77.1	58.5	12.6
G", kPa	19.9	14.6	4.16	25.9	18.4	4.7
η*, kPa·s	0.59	0.45	0.12	0.81	0.61	0.13
Shear stress, kPa	40.72	25.66	7.87	54.03	42.91	14.43
Shear strain, kPa	2.15	1.64	0.16	1.84	1.47	0.30
Shear rigidity	25.13	12.31	4.84	36.98	25.24	9.51

[1]Minimum significant difference

3.3 Meltability and rheology

Although the meltability of only one cheese type in- ɔreased significantly from 1 wk to 6 wk, the mean neltability of the fresh milk cheeses and of the reconsti- uted milk cheeses increased significantly during stor- ıge (Table 2). When α_{s1}-casein is degraded, the re- naining large peptides no longer interact with other ;aseins, causing the casein submicelles to reorganize ıto a more porous pattern, which weakens the protein natrix (18). The fat globules dispersed in the matrix hen coalesce, resulting in a more meltable cheese 19), which is a desirable characteristic for Mozzarella.

The cheeses made from reconstituted milk tended to ıave higher TPA hardness and lower TPA cohesive- ıess values than the others. The decrease in hardness ·xhibited by a cheese during storage was usually not ignificant, but the decrease in the mean value was sig- ificant for both the fresh milk cheeses and the reconsti- ıted milk cheeses. Increases in cohesiveness with torage were significant with every cheese except those ıade from milk with 2% added CC. Significant decreases /ere also observed in the mean values for G', G", and * during storage of the fresh milk cheeses and of the ɔconstituted milk cheeses. All of the values were within ıe normal range for commercial Mozzarella cheese.

The smaller peptides formed during proteolysis could easily move aside upon application of force (12), which would account for the reduction in the TPA hardness (the maximum force during the first compression) and small amplitude oscillatory shear values during refrigerated storage. The TPA cohesiveness (the ratio of the force area of the second compression to that of the first) increased because the force required for the second compression does not decrease greatly during storage. These effects have been previously observed with low fat (<10% fat w/w) Mozzarella prepared in this laboratory (19–21).

Stress at fracture and shear rigidity tended to decrease during storage, but the only the control displayed significant decreases in both. Cheeses made with reconstituted milk containing 9% NFDM continued to fracture at high stress even after 6 weeks of storage. The mean values of shear strain and shear rigidity significantly decreased during storage for both fresh milk and reconstituted milk cheeses, which is also attributable to proteolysis of α_{s1}-casein.

Texture attributes, defined as brittle, tough, mushy, or rubbery, can be analyzed in terms of stress vs strain (10). All cheeses became more rubbery with refrigerated storage except the cheese made with reconstituted milk containing 9% NFDM, which tended to become tougher with time (Fig. 1). Cheeses showed less variation in shear strain (gel cohesiveness) than in shear stress (gel strength). Cheeses made from milk with 1 or 2% added NFDM were more rubbery than cheese made from reconstituted NFDM. The reported lack of textural differences found in cheeses made with up to 3% added NFDM (25) are not in agreement with our results.

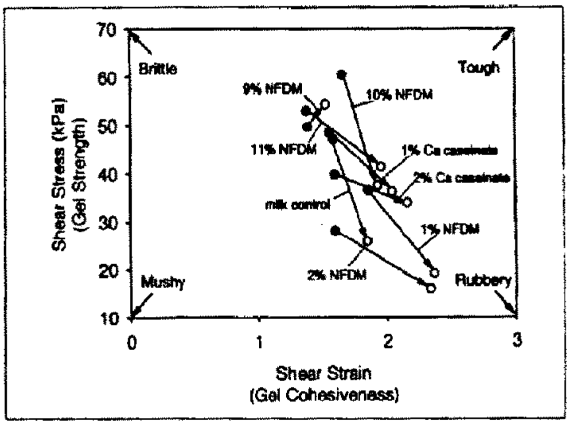

Fig. 1: Texture map (shear stress vs. shear strain) of changes in Mozzarella cheeses after 1 wk (●) and 6 wk (O) of refrigerated storage, as measured by torsion gelometry

The TPA hardness vs. cohesiveness relationship resembled the shear stress vs. strain relationship, as almost all samples became softer and more cohesive, *i.e.* more rubbery. A previous study on relationships between TPA and torsion gelometry in tests of 8 food protein gels showed that shear stress and TPA hardness correlated very strongly with each other, as did shear strain and TPA cohesiveness (5).

Torsion analyses and TPA deal with fracture of cheese at high strain levels, and provide different information than small amplitude oscillatory shear analyses, in which the specimen remains intact. Nevertheless, the mean values for hardness, cohesiveness, G', G", η*, shear strain, and shear rigidity for the fresh milk and reconstituted milk cheeses all showed significant changes during refrigerated storage. Further research now underway in our laboratory will deal with correlations between TPA, small amplitude oscillatory shear, and torsion analyses in cheese.

4. Conclusions

The fat content of part-skim Mozzarella cheese is reduced when CC is added to the cheese milk or when the cheese is made from reconstituted milk containing 11% NFDM. Proteolysis during refrigerated storage causes significant decreases in the α_{s1}-casein content in cheese made from fortified or reconstituted milk, resulting in increases in meltability and TPA cohesiveness, and decreases in TPA hardness, G', G", η*, shear strain, and shear rigidity. All but one cheese type became more rubbery during the 6-wk storage period. The textural and melting properties of Mozzarella cheese are not affected if CC and NFDM are added to milk at a concentration of 1 or 2%.

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6. References

(1) AOAC INTERNATIONAL: Method 948.12. In Official Methods of Analysis, 16th ed. AOAC International, Gaithersburg, MD (1997)
(2) FLANAGAN, J.F., THOMPSON, M.P., BROWER, D.P., GYURICSEK, D.M.: Cult. Dairy Prod. J. **23** 24–28 (1987)
(3) FOX, P.F.: J. Dairy Sci. **72** 1379–1400 (1989)
(4) GROVES, D.: Cheese Reporter **120** (11) 1 (1995)
(5) HAMANN, D.D.: Food Technol. **42** 66–71 (1988)
(6) HAMANN, D.D.: In Interactions of Food Proteins (Ed. N. Parris, R. Barford) Amer. Chem. Soc., Washington, DC (1991)
(7) KINDSTEDT, P.S.: Crit. Revs. Food Sci. Nutr. **33** 167–187 (1993)
(8) KJAERGAARD JENSEN, G., IPSEN, R.H., ILSØE, C.: Food Technol. **41** (10) 66–71 (1987)
(9) KOSIKOWSKI, F.V., MISTRY, V.V.: Cheese and Fermented Milk Foods. Vol. 2: Procedures and Analysis. 3rd ed. F.V. Kosikowski LLC, Westport, CT, 212, 259 (1997)
(10) LANIER, T.C.: In Rheology of Food Biopolymer Gels: A Practical Approach. North Carolina State University, Raleigh (1995)
(11) LAWRENCE, R.C., CREAMER, L.K., GILLES, J.: J. Dairy Sci. **70** 1748–1760 (1987)
(12) MALIN, E.L., BROWN, E.M.: In Chemistry of Structure-Function Relationships in Cheese (Ed. E.L. Malin, M.H. Tunick). Plenum, New York, 303–310 (1995)
(13) MAUBOIS, J.-L.: In Cheesemaking: Science and Technology (Ed. A. Eck) Lavoisier Publ., New York, NY, 156–164 (1987)
(14) McSWEENEY, P.L.H., OLSON, N.F., FOX, P.F., HEALY, A., HOJRUP, P.: J. Dairy Res. **60** 401-412 (1993)
(15) PEARSE, M.J., MACKINLAY, A.G.: J. Dairy Sci. **72** 1401–1407 (1989)
(16) SAS Institute: SAS/STAT User's Guide, Vers. 6, Vol. 2, 4th ed. Cary, NC (1989)
(17) TUNICK, M.H., BASCH, J.J., MALEEFF, B.E., FLANAGAN, J.F., HOLSINGER, V.H.: J. Dairy Sci. **72** 1976–1980 (1989)
(18) TUNICK, M.H., COOKE, P.H., MALIN, E.L., SMITH, P.W., HOLSINGER, V.H.: Int. Dairy J. **7** 149–155 (1997)
(19) TUNICK, M.H., MACKEY, K.L., SHIEH, J.J., SMITH, P.W., COOKE, P., MALIN, E.L.: Int. Dairy J. **3** 649–662 (1993)
(20) TUNICK, M.H., MALIN, E.L., SMITH, P.W., SHIEH, J.J., SULLIVAN, B.C., MACKEY, K.L., HOLSINGER, V.H.: J. Dairy Sci. **76** 3621–3628 (1993)
(21) TUNICK, M.H., MALIN, E.L., SMITH, P.W., HOLSINGER, V.H.: Int. Dairy J. **5** 483–491 (1995)
(22) TUNICK, M.H:, NOLAN, E.J.: In Physical Chemistry of Food Processes. 1 - Fundamental Aspects (Ed. I.C. Baianu) AVI Books, New York 273–297 (1992)
(23) USDA: Dairy Products 1998 Summary. US Government Printing Office, Washington DC, 35 (1999)
(24) USFDA: Code of Federal Regulations. US Government Printing Office, Washington, DC, Title 21, Section 133 (1998)
(25) YUN, J. J., BARBANO, D. M., LAROSE, K. L., KINDSTEDT, P.S.: J. Dairy Sci. **81** 1–8 (1998)